Ankara Univ Vet Fak Derg, **68**, 275-281, 2021
DOI: 10.33988/auvfd.708329

Molecular detection and phylogenetic analysis of *Theileria equi* and *Babesia caballi* in wild horses in Konya province of Turkey

Özlem DERİNBAY EKİCİ[1,a], Onur CEYLAN[1,b,✉], Gonca SÖNMEZ[2,c], Bilal DİK[1,d], Ceylan CEYLAN[1,e], Asma SEMASSEL[3,f]

[1]Selçuk University, Faculty of Veterinary Medicine, Department of Veterinary Parasitology, Konya, Turkey; [2]Selçuk University, Faculty of Veterinary Medicine, Department of Zootechnics and Husbandry, Konya, Turkey; [3]Selçuk University, Institute of Health Sciences, Konya, Turkey.

[a]ORCID: 0000-0002-0509-091X; [b]ORCID: 0000-0002-3514-5221; [c]ORCID: 0000-0002-4946-3749; [d]ORCID: 0000-0002-7553-5611; [e]ORCID: 0000-0001-8072-2983; [f]ORCID: 0000-0002-6657-8644

✉Corresponding author: onurceylan@selcuk.edu.tr
Received date: 11.08.2020 - Accepted date: 27.11.2020

Abstract: The aim of this study was to investigate equine piroplasms of wild horses (*Equus ferus caballus*) in Konya province of Turkey in November-December 2017. For this aim, blood samples were collected from 36 wild horses and examined for equine piroplasms by microscopy and multiplex PCR. Some of the PCR products from positive samples were also sequenced. Five (13.89%) out of the 36 horses were infected with either *Theileria equi, Babesia caballi* or both in the microscopical examination. Single infections with *T. equi* and *B. caballi* were detected in three (8.33%) and one horses (2.78%), respectively. Prevalence of *T. equi*, *B. caballi* and mix infections was determined as 50%, 38.8% and 38.8% by multiplex PCR, respectively. Multiplex PCR was found more sensitive than microscopical examination to detect the piroplasms of horses. The results of sequence analysis showed 99.25-100% and 98.23-99.59% nucleotide sequence identity to the previously reported *T. equi* and *B. caballi* 18S rRNA gene sequences, respectively. Consequently, the existence of equine piroplasmosis in wild horses was reported for the first time in Turkey, and high molecular prevalences of *T. equi* and *B. caballi* were reported with this study.

Keywords: *Babesia caballi,* multiplex PCR, *Theileria equi,* Turkey, wild horse.

Türkiye'nin Konya yöresinde yaban atlarında *Theileria equi* ve *Babesia caballi*'nin moleküler tespiti ve filogenetik analizi

Özet: Bu çalışma Türkiye'nin Konya yöresinde yaban atlarının (*Equus ferus caballus*) equine piroplasmlarını tespit etmek amacıyla Kasım-Aralık 2017 tarihlerinde yürütülmüştür. Bu amaçla, 36 yaban atına ait kan örnekeri toplanmış ve bu örnekler mikroskobi ve multiplex PCR yöntemi ile equine piroplasmlar yönünden incelenmiştir. Ayrıca pozitif olduğu tespit edilen bazı örneklere ait PCR ürünleri sekanslanmıştır. Mikroskobik incelemede 36 atın 5'i (13,89%) *Theileria equi, Babesia caballi* veya her ikisiyle birlikte enfekte bulunmuştur. Üç (8,33%) atta *T. equi*, bir (2,78%) atta da *B. caballi* tek tür olarak tespit edilmiştir. *T. equi* ve *B. caballi* ve miks enfeksiyonların prevalansları multiplex PCR ile sırasıyla %50, %38,8 ve %38,8 olarak belirlenmiştir. Çalışmada kan parazitlerinin tespitinde multiplex PCR yönteminin mikroskobik incelemeden daha duyarlı olduğu görülmüştür. Sekans analizleri sonucunda *T. equi* ve *B. caballi* 18S rRNA genleri ile sırasıyla 99,25-100% ve 98,23-99,59%'luk benzerlik tespit edilmiştir. Bu çalışma Türkiye'deki yaban atlarında equine piroplasmosis'in varlığını bildiren ilk çalışmadır ve çalışmada *T. equi* ve *B. caballi*'nin moleküler prevalansları yüksek düzeyde tespit edilmiştir.

Anahtar sözcükler: *Babesia caballi,* multipleks PCR, *Theileria equi,* Türkiye, yaban atı.

Introduction

The origin of domestic horses on the earth is wild horses known as Przewalski and Tarpan horses which are now extinct. But, even today, Przewalski horses (*Equus ferus przewalskii*) (L.S. Poliakov, 1881) live in the forest of Siberia and Mongolia. The Tarpan horse (*Equus ferus ferus*) (Boddaert, 1785), is a subspecies of wild horse and known as Eurasian wild horse. Wild horses are known as "Yılkı horse" in Anatolia (3).

Theileria equi and *Babesia caballi*, the main agents of equine piroplasmosis, are transmitted by ixodid ticks (18). Equine piroplasmosis cause severe symptoms

including fever, anemia, hemoglobinuria, icterus, anorexia, thrombocytopenia, tachypnea tachycardia, loss of appetite, and petechial haemorrhages on mucous membranes (51). The pathogenicity and prevalence of *T. equi* is higher than *B. caballi* in endemic countries (15). Diagnosis of equine piroplasmosis is traditionally made by the detection of piroplasms in Acridine Orange or Giemsa stained peripheral thin blood smears in microscopical examination (45). However, in the case of low parasitemia and mixed infections, microscopy is not sufficient for accurate identification of equine piroplasms (36, 43). Serological tests such as Enzyme-Linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT), Indirect Fluorescent Antibody Test (IFAT) and Western-blotting (14, 16, 27, 29, 32, 37); and molecular tests like Polymerase Chain Reaction (PCR), Nested PCR and Real Time PCR could be used for the detection of latent and subclinical infections (13, 20). *Babesia* species were detected by PCR in cattle and horses in many studies (10, 17, 21, 22). In the studies conducted with ELISA, IFAT, and CFT in horses in Turkey, the seroprevalences of *T. equi* and *B. caballi* were varied between 0-100% and 0-56.9%, respectively (1, 2, 7, 30, 31, 38, 41, 46). However, the molecular prevalence of *B. caballi* and *T. equi* was detected between 1.97-3% and 2.96-44.6%, respectively in PCR (19, 24, 25, 35, 42). All the studies above were carried out in domestic horses and no data is available on the *Theileria equi* ve *Babesia caballi* of wild horses in Turkey.

This study was aimed to detect the etiological agents of equine piroplasmosis of wild horses in Konya province of Turkey, to provide a molecular characterization of the isolates.

Materials and Methods

***Study area, sample collection, and microscopic examination*:** This study was conducted on the wild horses (*Equus ferus caballus*) brought to Karkın village in Konya (Figure 1) from Karadağ mountain in Karaman in Turkey, between November-December 2017. A total of 36 wild horses caught by the Kazakh horse herdsmen were examined for equine piroplasms. The thin blood smears for each horse were prepared and stained by Giemsa and examined in a light microscope (Leica DM 1000).

***Ethical statement*:** To carry out all procedures in this study, ethical guidelines for the use of animal samples permitted by Selçuk University, Veterinary Medicine (Permit for an animal experiment: 2018/05, Date: 13.02.2018) were tracked.

DNA extraction: 10 ml of blood samples were taken from each horse into tubes including EDTA for molecular studies and all horses were also visually examined in terms of tick infestations. 200 µl of blood samples was utilized for total genomic DNA (gDNA) isolation, using a commercial kit (Quick-DNA Miniprep Plus Kit, Zymo Research D4068). The isolated gDNA was stored at -20ºC until use.

PCR amplification: To detect *T. equi* and *B. caballi*, species-specific primers targeting 18S rRNA genes of these piroplasms were employed. The protocol of Alhassan et al. (4) was used for multiplex PCR. Each reaction consisted of 4 µl of gDNA and 46 µl of PCR mix containing 1.5 mM $MgCl_2$, 30 mM KCl, 250 µM of each dNTP, 10 pmol of each reverse primers for *T. equi* (EquiR:5-TGCCTTAAACTTCCTTGCGAT-3) and for *B. caballi* (CabR:5-CTCGTTCATGATTTAGAATTGC-3), 20 pmol of universal forward primer (UFP:5-TCGAAGACGATCAGATACCGTCG-3) and 1 U of Taq polymerase (Bioline). BioRad thermocycler was used for the reactions with the following programme: 96ºC for 10 min, 36 cycles (96ºC for 1 min, 60.5ºC for 1 min, 72ºC for 1 min) and final extension 72ºC for 10 minutes. As a negative control, distilled water was used. The amplified DNA samples electrophoresed on agarose gel (1%) in TAE buffer, were stained with ethidium bromide and then photographed using UV transilluminator.

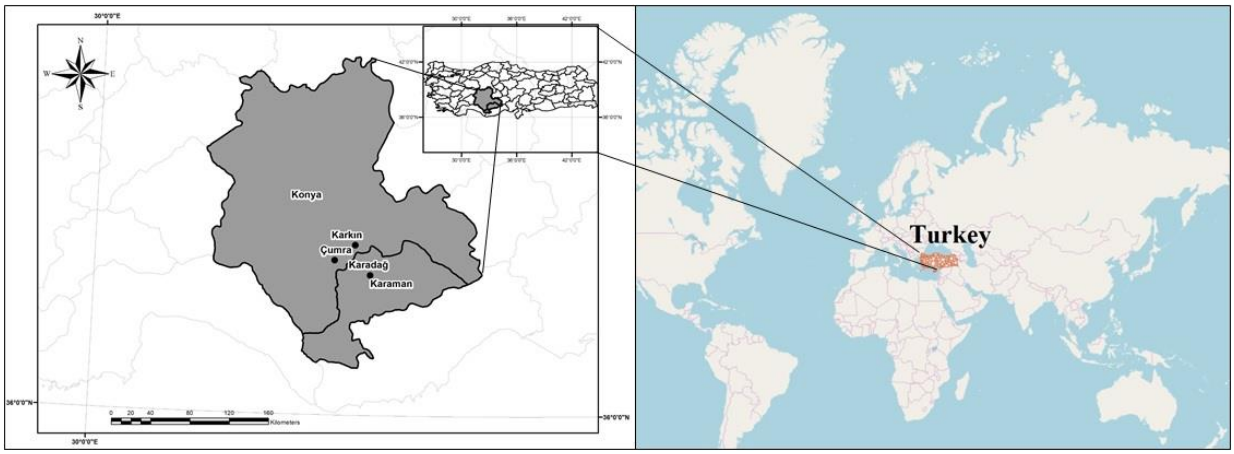

Figure 1. The geographic location of sample collection site.

Phylogenetic analysis: Eight positive products were randomly selected and sequenced. Sequences were analyzed by using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic trees were constructed by using the neighbor-joining method in MEGA version X software generated from the 18S rRNA on aligned sequences of the *T. equi* and *B. caballi* 18S rRNA gene sequences.

Results

As a result of microscopic examination, 5 (13.89%) of the horses were found positive for equine piroplasms. *Theileria equi* in three cases (8.33%), and *B. caballi* in one case (2.78%) were detected as infections with single species in the microscopical examination. Mix infections with these parasites were found in one case (2.78%).

Theileria equi and *B. caballi* were found positive in 18 (50%) and 14 (38.8%) cases, respectively by multiplex PCR. Mix infections were found in 14 cases (38.8%). As a result of the multiplex PCR assay, the presence of bands of 435 bp and 584 bp was determined for *T. equi* and *B. caballi*, respectively (Figure 2). *Theileria equi* was found more prevalent than *B. caballi.* Microscopy and multiplex PCR results are summarized in Table 1.

Theileria equi isolates obtained in this study formed a well-supported clade with the sequences from Turkey, Sudan, South Africa, Brazil, Romania, China, Spain, Switzerland, Jordan, and the USA (Figure 3). The sequences showed 99.25-100% nucleotide sequence identity with the sequences from these countries.

Table 1. Microscopy and multiplex PCR results.

Piroplasm species	Microscopy	Multiplex PCR
B. caballi	1	-
T. equi	3	4
B. caballi + *T. equi*	1	14
Total	5	18

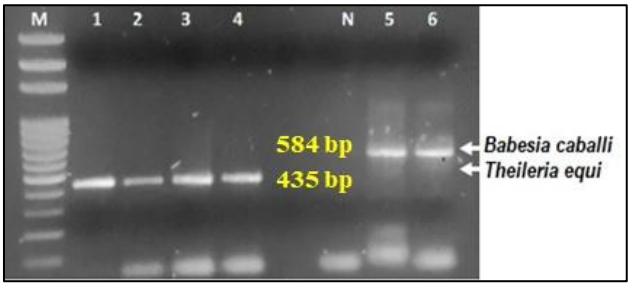

Figure 2. PCR detection of *B. caballi* and *T. equi* with a set of primers. M: 100 bp DNA marker; N: Negative control; Lane 1-4: *T. equi*; Lane 5-6 *B. caballi.*

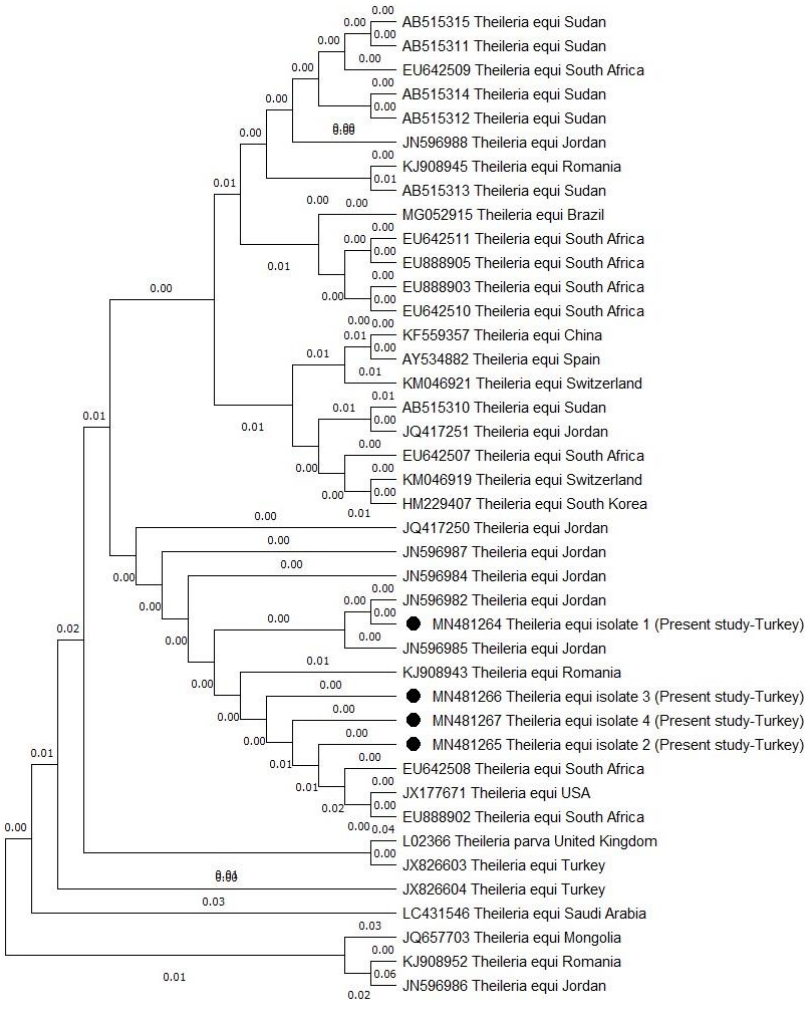

Figure 3. Phylogenetic tree constructed by using the neighbor-joining method in MEGAX software generated from the 18S rRNA on aligned sequence of the *T. equi* 18S rRNA gene. GenBank accession numbers of reference sequences and localities are given. The sequences obtained from this study were marked with the symbol as " •".

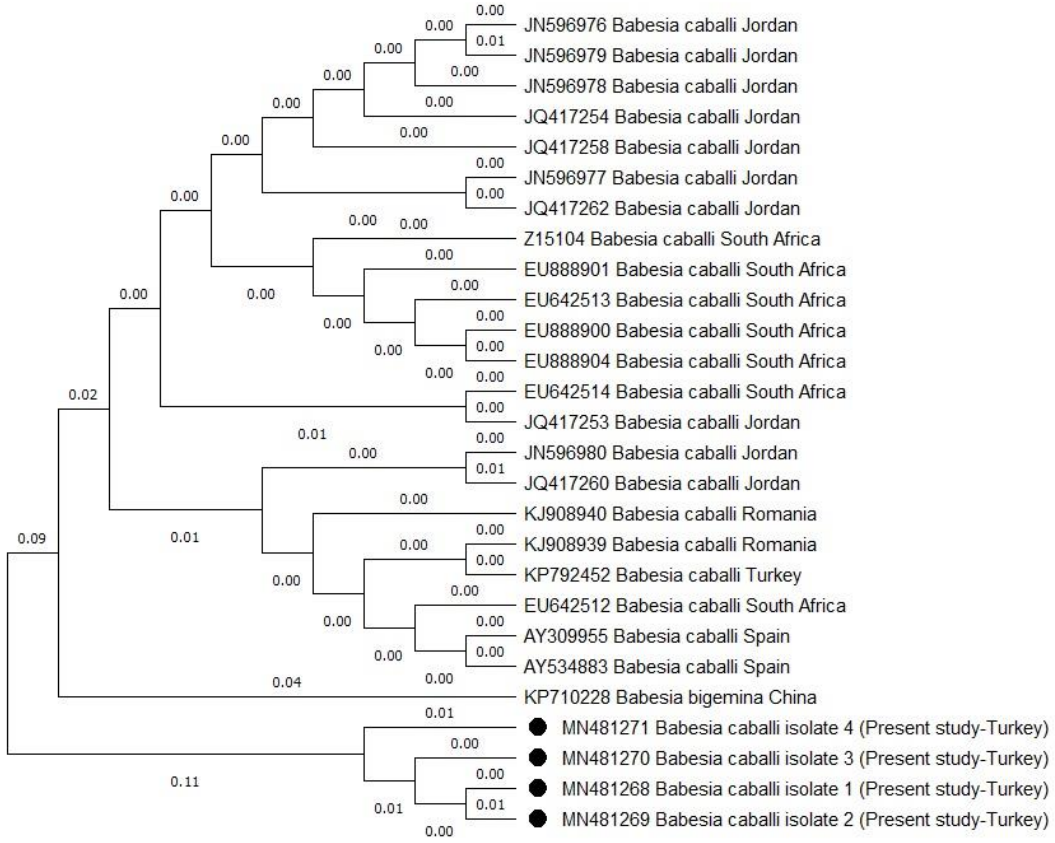

Figure 4. Phylogenetic tree constructed by using the neighbor-joining method in MEGAX software generated from the 18S rRNA on aligned sequence of the *B.caballi* 18S rRNA gene. GenBank accession numbers of reference sequences and localities are given. The sequences obtained from this study were marked with the symbol as " •".

However, *B. caballi* isolates obtained in the present study clustered together in a separate clade (Figure 4) with high proximity to other sequences in the different clades. The nucleotide sequence identity value of *B. caballi* 18S rRNA gene sequences ranged from 98.23-99.59%. The obtained sequences were deposited to Genbank under the accession numbers MN481268-MN481271 for *B. caballi* and MN481264-MN481267 for *T. equi.*

Discussion and Conclusion

So far, only one report (33) on the parasites of wild horses was encountered in Turkey reporting helminth infections. However, no study has been conducted on the *T. equi* ve *B. caballi* in wild horses in Turkey.

Piroplasmosis is a serious disease for all horses, but it is even more crucial for racing horses. The international movement of racing horses increases the probability of disease transmission from infected horses to susceptible ones (23). Previous studies on the diagnosis of piroplasmosis focused on microscopic examination of Giemsa stained preparations. The prevalence of equine piroplasmosis was found to vary between 0-58.18% in Turkey (2, 7, 25, 31, 38). In our study, 5 out of 36 horses were found positive for equine piroplasms, microscopically. *Theileria equi* and *B. caballi* were detected as a single species in three and one cases, respectively and both species were found together in one case. In microscopical examination, the infection rates of *T. equi* and *B. caballi* obtained in this study were found to be higher than the previously reported ones (7, 38). However, the infection rate of *B. caballi* was lower than the result of a previous study conducted in the Central part of Turkey (31). In the microscopy, the low infection rate of *B. caballi* in this study may be due to the latent course of the infection or the season of the study. Since this study was carried out in late autumn and early winter, it is very difficult to find piroplasms in the erythrocytes of the horses in this period of the year. In addition, vector ticks, except *Haemaphysalis* and *Dermacentor* species are inactive in this season in Central Anatolia. The blood samples taken from the vena jugularis may also be another cause of low parasitemia. Microscopic examination may be insufficient in the latent period when parasites are present in the blood in small numbers (14, 28, 47). Stress factors are very important in this infection, and they may temporarily affect the immune system. Some studies

indicating that immunosuppressed animals are more susceptible to infection (28, 34). Since these horses live a relatively remote life from stress in their natural environment, there are no or few conditions that would adversely affect their immune system. In the detection of parasites in the cases of low parasitemia, more sensitive and specific diagnostic techniques such as molecular methods are preferred (12, 18, 26). 18S rRNA gene has been extensively used for the phylogenetic analysis of piroplasms (5). Many researchers stated that the results of PCR based molecular studies were much more sensitive compared to those of microscopic examination (10, 39, 40, 44, 48, 49). In this study, it was also determined that 18S rRNA based multiplex PCR was more sensitive than microscopy in the detection of equine piroplasms. The prevalence of *T. equi* and *B. caballi* was found as 50% and 38.8%, respectively by PCR. The obtained results of the study support the findings of the previously conducted studies in South Africa and Brazil (12, 18, 26) mentioned above that PCR is more sensitive than microscopy. Fifteen blood samples negative in microscopical examination, were *T. equi*-positive in PCR, whereas one microscopy positive sample was negative in PCR. Twelve blood samples, found as negative for *B. caballi* in microscopical examination, were positive in PCR. On the other hand, two *B. caballi* microscopy-positive samples were also positive in PCR. Negative detection in the PCR of one sample found to be *T. equi*-positive on microscopy may be referred to the existence of PCR inhibitors or parasitemia lower than the detection limit of the molecular technique in the circulating blood of hosts (6, 8, 9, 11). The sequencing results obtained from this study were similar to those of equine piroplasms genotypes previously registered to Genbank.

The number of molecular studies on equine piroplasmosis are restricted in Turkey. In these studies, prevalences of *B. caballi* and *T. equi* have been reported as follows: 3% and 7% in PCR; 2.3% and 44.6% in nested PCR; 1.97% and 2.96% in Real Time PCR, respectively (19, 24, 35). In the other studies conducted in Turkey, Guven et al. (25) detected *T. equi* (8.8%) by multiplex PCR in Arabian horses in Erzurum province. Also, Ozubek and Aktas (42) reported the piroplasm infection rate of 33.5% in equids by RLB. No *B. caballi* infection was detected in sampled horses in both of these studies. In the current study, *T. equi* prevalence was 50%, while of *B. caballi* was found as 38.8% in multiplex PCR. These results were higher than the results of the previous studies (24, 35). This may be due to the fact that wild horses have been exposed to continuous tick infestations in nature without veterinary control and treatment. Preimmune horses are reservoirs for healthy horses and therefore infection persists continuously in wild horses.

The etiological agents of equine piroplasmosis are transmitted by some ixodid tick species in the genera of *Rhipicephalus, Hyalomma* and *Dermacentor* (18, 33, 50). *Rhipicephalus* and *Hyalomma* species are seen between spring-autumn in Central Anatolia. Vector tick species of equine piroplasmosis in Turkey are not known well. Four tick species: *Hy. detritum, Hy. marginatum, Rh. turanicus* and *Rh. bursa* were determined in the horses with equine piroplasmosis in the previous studies in Turkey (1, 2, 30, 31). Vector tick species have not been studied in detail in most of those studies on determination of the presence and/or prevalence of equine piroplasmosis.

In conclusion; the wild horses were systematically studied for equine piroplasmosis by using microscopical and molecular techniques for the first time in Turkey. *Theileria equi* was found to be more common than *B. caballi.* The specificity of multiplex PCR was higher than microscopic examination. Wild horses live in nature without veterinary control and these horses are constantly a source of infection for domestic horses. Therefore, further studies are needed to detect the prevalence of equine piroplasms and their relationship with vectors throughout the country.

Acknowledgement

The authors would like to thank Professor Zeki Ogurtan for English editing.

Financial Support

Financial support for the present study was provided by Selcuk University Scientific Research Projects Coordination Office (Project No: 18401042).

Ethical Statement

This study was approved by the Selçuk University Animal Research Ethics Committee, (Permit for animal experiment: 2018/05, Date: 13.02.2018).

Conflict of Interest

The authors declared that there is no conflict of interest.

References

1. **Acici M, Umur S, Guvenc T, et al** (2008): *Seroprevalence of equine babesiosis in the Black Sea region of Turkey*. Parasitol Int, **57**, 198-200.
2. **Akkan HA, Karaca M, Tutuncu M, et al** (2003): *Serologic and microscopic studies on babesiosis in horses in the Eastern border of Turkey*. J Equine Vet Sci, **23**, 181–183.
3. **Aksoy AR** (2016): At yetiştiriciliği ders notları. Available at https://docplayer.biz.tr/22854819-At-yetistiriciligi-ders-notlari-yrd-doc-dr-ali-riza-aksoy-1993-guncelleme-mayis-2016.html. (Accessed March 26, 2020).

4. **Alhassan A, Pumidonming W, Okamura M, et al** (2005): *Development of a single round and multiplex PCR method for the simultaneous detection of Babesia caballi and Babesia equi in horse blood*. Vet Parasitol, **129**, 43–49.
5. **Allsopp MT, Allsopp BA** (2006): *Molecular sequence evidence for the reclassification of some Babesia species*. Ann N Y Acad Sci, **1081**, 509–517.
6. **Allsopp MTEP, Lewis BD, Penzhorn BL** (2007): *Molecular evidence for transplacental transmission of Theileria equi from carrier mares to their apparently healthy foals*. Vet Parasitol, **148**, 130–136.
7. **Balkaya I, Erdogmus Z** (2006): *Investigation of prevalence of Babesia equi (Laveran, 1901) and Babesia caballi (Nuttall 1910) in horses by serological methods in Elazığ and Malatya province*. Fırat Üniv Sag Bil Derg, **20**, 61–63.
8. **Baptista AP, Franco C, Mendes M, et al** (2010): *Subunit composition of Rhodothermus marinus respiratory complex I.* Anal Biochem, **407**, 104–110.
9. **Baptista C, Lopes MS, Tavares AC, et al** (2013): *Diagnosis of Theileria equi infections in horses in the Azores using cELISA and nested PCR.* Ticks Tick Borne Dis, **4**, 242–245.
10. **Bashiruddin JB, Cammà C, Rebêlo E** (1999): *Molecular detection of Babesia equi and Babesia caballi in horse blood by PCR amplification of part of the 16s rRNA gene.* Vet Parasitol, **84**, 75–83.
11. **Bhoora R** (2009): Molecular characterization of Babesia caballi and Theileria equi, the aetiological agents of equine piroplasmosis, in South Africa. PhD. Thesis. University of Pretoria, South Africa.
12. **Bhoora R, Quan M, Franssen L, et al** (2010): *Development and evaluation of real-time PCR assays for the quantitative detection of Babesia caballi and Theileria equi infections in horses from South Africa*. Vet Parasitol, **168**, 201-211.
13. **Boldbaatar D, Xuan X, Battsetseg B, et al** (2005): *Epidemiological study of equine piroplasmosis in Mongolia*. Vet Parasitol, **127**, 29-32.
14. **Bose R, Jorgensen WK, Dalgliesh RJ, et al** (1995): *Current state and future trends in the diagnosis of babesiosis*. Vet Parasitol, **57**, 61-74.
15. **Bruning A** (1996): *Equine piroplasmosis: an update on diagnosis, treatment and prevention.* Br Vet J, **152**, 139-151.
16. **Bruning A, Phipps P, Posnett E, et al** (1997): *Monoclonal antibodies against Babesia caballi and Babesia equi and their application in serodiagnosis*. Vet Parasitol, **58**, 11-26.
17. **Calder JA, Reddy GR, Chieves LP, et al** (1996): *Monitoring Babesia bovis infections in cattle by using PCRbased tests*. J Clin Microbiol, **11**, 2748-2755.
18. **De Waal DT** (1992): *Equine piroplasmosis: A review*. Br Vet J, **148**, 6-14.
19. **Deniz A, Karaer Z, Cakmak A, et al** (2007): Türkiye'de atlarda Babesia etkenlerinin saptanmasında PCR tekniğinin uygulanması (TAGEM, Project number: HS/01/02/06/05/103).
20. **Edwards RZ, Moore H, LeRoy BE, et al** (2011): Equine Babesiosis. Available at http://www.vet.uga.edu/vpp/clerk/edwards/index.php. (Accessed October 15, 2019).
21. **Fahrimal Y, Gott WL, Jamer DP** (1992): *Detection of Babesia bovis carrier cattle by using polymerase chain reaction amplification of parasite DNA*. J Clin Microbiol, **30**, 1374-1379.
22. **Figueroa JV, Chieves LP, Johnson GS, et al** (1993): *Multiplex polymerase chain reaction based assay for the detection of Babesia bigemina, Babesia bovis, and Anaplasma marginale DNA in bovine blood*. Vet Parasitol, **50**, 69-81.
23. **Friedhoff KT** (1988): Transmission of Babesia. 23-52. In: M Ristic (Ed), Babesiosis of Domestic Animals and Man. CRC Press, Boca Raton FL.
24. **Guclu HZ, Karaer Z** (2007): *Detection of Babesia caballi (Nuttall, 1910) and Theileria equi (Syn. Babesia equi, Laveran, 1901) by the Polymerase Chain Reaction (PCR) in show and sport horses in the region of Ankara*. Türkiye Parazitol Derg, **31**, 89-93.
25. **Guven E, Avcioglu H, Deniz A, et al** (2017): *Prevalence and molecular characterization of Theileria equi and Babesia caballi in jereed horses in Erzurum, Turkey*. Acta Parasitol, **62**, 207-213.
26. **Heim A, Passos LMF, Ribeiro MFB, et al** (2007): *Detection and molecular characterization of Babesia caballi and Theileria equi isolates from endemic areas of Brazil*. Parasitol Res, **102**, 63-68.
27. **Hirata H, Xuan X, Yokoyama N, et al** (2003): *Identification of a specific antigenic region of the P82 protein of Babesia equi and its potential use in serodiagnosis.* J Clin Microbiol, **41**, 547-551.
28. **Hodgson J** (2002): Equine exotic diseases, a manual for horse owners. Union Offset, Canberra.
29. **Ikadai H, Osorio CR, Xuan X, et al** (2000): *Detection of Babesia caballi infection by enzyme-linked immunosorbent assay using recombinant 48-kDa merozoite rhoptry protein*. Int J Parasitol, **30**, 633-635.
30. **Inci A** (1997): *Detection of Babesia caballi (Nuttall, 1910) and Babesia equi (Laveran, 1901) in horses by microscopic examination in military farm in Gemlik.* Turk J Vet Anim Sci, **21**, 43-46.
31. **Inci A** (2002): *Investigation of the prevalence of Babesia equi (Laveran, 1901) and Babesia caballi (Nuttall, 1910) in equids by microscopic examination in Kayseri region*. Fırat Üniv Sag Bil Derg, **1**, 85-88.
32. **Kappmeyer LS, Perryman LE, Hines SA, et al** (1999): *Detection of equine antibodies to Babesia caballi by recombinant B. caballi rhoptry-associated protein 1 in a competitive-inhibition enzyme-linked immunosorbent assay*. J Clin Microbiol, **37**, 2285-2290.
33. **Karaman Il Cevre ve Orman Mudurlugu** (2010): Karadağ mevkiindeki Yılkı atlarının durum tespiti, genel sağlık durumları, üremelerinin kontrolü ve çevre üzerindeki etkilerinin değerlendirilmesi projesi. Mevlana Kalkınma Ajansı Teknik Destek Projesi, Referans No: TR52-10-TD01.
34. **Kerber CE, Ferreira F, Pereira MC** (1999): *Control of equine piroplasmosis in Brazil*. Onderstepoort J Vet Res, **66**, 123-127.
35. **Kizilarslan F, Yildirim A, Duzlu O, et al** (2015): *Molecular detection and characterization of Theileria equi and Babesia caballi in horses (Equus ferus caballus) in Turkey*. J Equine Vet Sci, **35**, 830-835.

36. **Krause PJ** (2003): *Babesiosis diagnosis and treatment*. Vector Borne Zoonotic Dis, **3**, 45-51.
37. **Kumar S, Kumar Y, Malhotra DV, et al** (2003): *Standardization and comparison of serial dilution and single dilution elisa using different antigenic preparations of the Babesia equi parasite*. Vet Res, **34**, 71-83.
38. **Kurt C, Yaman M** (2005): *The investigation of the prevalence Babesia equi and Babesia caballi in horses by microscopic and serologic (cELISA) methods in Adana province*. YYU Vet Fak Derg, **23**, 1-4.
39. **Moretti A, Mangili V, Salvatori R, et al** (2010): *Prevalence and diagnosis of Babesia and Theileria infections in horses in Italy: A preliminary study*. Vet J, **184**, 346-350.
40. **Nicolaiewsky TB, Richter MF, Lunge VR, et al** (2001): *Detection of Babesia equi (Laveran, 1901) by nested polymerase chain reaction*. Vet Parasitol, **101**, 9-21.
41. **Oncel T, Vural G, Gicik Y, et al** (2007): *Detection of Babesia (Theileria) equi (Laveran 1901) in Horses in the Kars Province of Turkey*. Türkiye Parazitol Derg, **31**, 170-172.
42. **Ozubek S, Aktas M** (2018): *Genetic diversity and prevalence of piroplasm species in equids from Turkey*. Comp Immunol Microbiol Infect Dis, **59**, 47-51.
43. **Quintao-Silva MG, Ribeiro MF** (2003): *Infection rate of Babesia spp. sporokinetes in engorged Boophilus microplus from an area of enzootic stability in the State of Minas Gerais, Brazil*. Mem Inst Oswaldo Cruz, **98**, 999-1002.
44. **Rampersad J, Cesar E, Campbell MD** (2003): *A field evaluation of PCR for the routine detection of Babesia equi in horses*. Vet Parasitol, **114**, 81-87.
45. **Saal JR** (1964): *Giemsa stain for the diagnosis of bovine babesiosis. II. Changes in erythrocytes infected with Babesia bigemina and B. argentina*. J Protozool, **11**, 582-585.
46. **Sari B, Kirmizigul AH, Deniz A, et al** (2009): *Seroprevalence of Babesia caballi and Theileria equi in horses from Kars and Ardahan provinces during the winter season*. Kafkas Üniv Vet Fak Derg, **16**, 657-661.
47. **Seifi HA, Mohri M, Sardari KA** (2000): *Mixed Infection of Babesia equi and Babesia caballi in a racing colt: a report from Iran*. J Equine Vet Sci, **20**, 858-860.
48. **Sloboda M, Jirků M, Lukešová D, et al** (2011): *A survey of piroplasmids in horses and Bactrian camels in North-Eastern Mongolia*. Vet Parasitol, **179**, 246-249.
49. **Tamzali Y** (2013): *Equine piroplasmosis: an updated review*. Equine Vet Educ, **25**, 590-598.
50. **Uilenberg G** (2006): *Babesia-A historical overview*. Vet Parasitol, **138**, 3-10.
51. **Wise LN, Kappmeyer LS, Mealey RH, et al** (2013): *Review of equine piroplasmosis*. J Vet Intern Med, **27**, 1334-1346.